

20. The method of claim 19, further comprising the step of adding to said sample, contemporaneously with antigen contact, a costimulus of T cell activation.

21. The method of claim 20, wherein said costimulus is an antibody specific for CD28.

22. The method of claim 20, wherein said costimulus is an antibody specific for VLA-4.

23. The method of claim 19, further comprising contacting said sample with an antibody specific for a T lymphocyte early activation antigen, and then flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset that concurrently bind said early activation antigen-specific antibody.

24. The method of claim 23, wherein said T lymphocyte early activation antigen is CD69.

25. The method of claim 19, further comprising the step, after adding said inhibitor of cytokine secretion and before flow cytometric detection, of permeabilizing said cells.

26. The method of any one of claims 19, 20, 23, or 25 wherein said sample is a whole blood sample.

27. The method of claim 26, further comprising the step of adding a cationic chelator after antigen contact is complete but prior to flow cytometric detection.

28. The method of claim 27, wherein said chelator is EDTA or EGTA.

29. The method of claim 28, wherein said chelator is EDTA.

30. The method of claim 26, further comprising the step of lysing red blood cells.

31. The method of claim 19, wherein said MHC-dependent nominal antigen is selected from the group consisting of alloantigens, viral antigens, autoantigens, viral antigens, and bacterial antigens.

32. The method of claim 31, wherein said MHC-dependent nominal antigen is a viral antigen.

33. The method of claim 32, wherein said antigen is a CMV antigen.

34. The method of claim 32, wherein said antigen is an HIV antigen.

35. The method of claim 32, wherein said antigen is a mumps antigen.

36. The method of claim 32, wherein said antigen is a measles antigen.

37. The method of claim 31, wherein said MHC-dependent nominal antigen is a bacterial antigen.

38. The method of claim 37, wherein said antigen is a *Mycobacterium tuberculosis* antigen.

39. The method of claim 19, wherein said inhibitor of cytokine secretion is Brefeldin A.

40. The method of claim 19, wherein said cytokine-specific antibody is specific for a cytokine selected from the group consisting of: IL-2, IL-4, IL-13, γ -IFN, and TNF- α .

41. The method of claim 40, wherein said cytokine-specific antibody is specific for IL-2.

42. The method of claim 40, wherein said cytokine-specific antibody is specific for IL-4.

43. The method of claim 40, wherein said cytokine-specific antibody is specific for γ -IFN.

44. The method of claim 40, wherein said cytokine-specific antibody is specific for TNF- α .

45. The method of claim 19, wherein said T lymphocyte subset-defining antibody is selected from the group consisting of antibodies specific for: CD3, CD4, CD8, TCR, homing receptors, CD45RO, CD45RA and CD27.

46. The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD3.

47. The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD4.

48. The method of claim 45, wherein said T lymphocyte subset-defining antibody is specific for CD8.

49. The method of any one of claims 19, 20, or 23 wherein said anti-cytokine antibodies, said T lymphocyte subset-defining antibodies, and said early activation antigen-specific antibodies are each conjugated directly to fluorophores.

50. The method of claim 49, wherein said fluorophores are selected from the group consisting of FITC, PE, PerCP, and APC.

51. The method of claim 50, wherein said anti-cytokine antibodies are conjugated to FITC.

52. The method of claim 50, wherein said T lymphocyte subset-defining antibodies are conjugated to PerCP.

53. The method of claim 50, wherein said antibody specific for a T lymphocyte early activation antigen is conjugated to PE.

54. The method of any one of claims 19, 20, or 23 wherein said antigen-contacting step lasts no longer than 24 hours.

55. The method of claim 54, wherein said antigen-contacting step lasts no longer than 6 hours.

56. A method of detecting memory/effector T lymphocytes that respond specifically to a vaccine antigen, comprising the steps, in order, of:

contacting a sample containing peripheral blood mononuclear cells with an MHC-dependent nominal vaccine antigen;

adding to said sample an inhibitor of cytokine secretion;

adding to said sample at least one cytokine-specific antibody and an anti-CD4 antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by CD4⁺ T lymphocytes.

57. A method of assessing CD4⁺ T cell effector frequencies in an HIV⁺ subject, comprising:

contacting a sample of said subject's peripheral blood mononuclear cells with an MHC-dependent nominal antigen;

adding to said sample an inhibitor of cytokine secretion;

adding to said sample at least one cytokine-specific antibody and an anti-CD4 antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by CD4⁺ cells.

58. A method of assessing the immunomodulatory effects of a chemical compound, comprising:

contacting a sample of whole blood with an MHC-dependent nominal antigen in the presence of said chemical compound;

adding to said sample an inhibitor of cytokine secretion;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

59. The method of claim 58, wherein the sample of whole blood is obtained from a human or animal treated with an immunosuppressive or an immunomodulatory compound.

60. The method of claim 59, wherein said compound is immunosuppressive.